

Fachbereich Veterinärmedizin
der Freien Universität Berlin
Fachrichtung Mikrobiologie

Research on Incidences and Isolation Methods of Salmonella from Faecal Samples of Naturally Infected Dogs

Inaugural-Dissertation

zur

Erlangung des Grades eines
Doktors der Veterinärmedizin
an der

Freien Universität Berlin

vorgelegt von

Habte Tesfamariam

Tierarzt aus Asmara

Berlin 1973

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Vorsitzender:	Prof. Dr. H.-J. Sinell
Referent:	Prof. Dr. H. Hartwigk
Korreferent:	Prof. Dr. L. Felix Müller



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I. INTRODUCTION

Salmonellosis as a zoonosis has become a world-wide problem, and it is believed that disease caused by non-typhoid *Salmonella* infection is increasing in both animal and man.

Until the present time, most interest has been given to egg and meat products as vehicles for human infection, while in spite of the nature of its association with man as a pet little attention has been paid to the role of the dog in the epidemiology of human salmonellosis. Most outbreaks of food poisoning in man are usually traceable to a specific infected food, in which infection from the dog is unlikely. It is of interest therefore to compare the range of serotypes isolated from dogs in this study with those isolated from man in hospitals.

To achieve an appropriate analysis of such incidence, the researcher found it to be advisable to use a suitable method and applicable material for the isolation of *Salmonella*. A number of enrichment and selective media have been used by various workers for the isolation of *Salmonella* organisms from faeces, and various claims have been made for the superiority of one or the other medium. In view of the diversity of opinion existing in literature on the isolation of *Salmonella*, it is considered desirable to carry out competitive trials with commonly employed enrichment broths and selective media to evaluate their comparative efficiency for the isolation of *Salmonella* from faeces.

MOSSEL and RATTO 1970 discussing the rapid detection of sublethally impaired cells of Enterobacteriaceae in dried food found out that in food processing the micro-organisms are sublethally inhibited by heat and food processing chemicals. The researcher believes that the same thing could happen to the micro-organisms in the faeces of dogs due to the gastro-

intestinal acidities and the antibiotics and sulphonamides used as therapy in clinics. Consequently, these injured cells need restoration treatment (resuscitation period) to begin active growth prior to their exposure to selective media currently used for their detection.

II. L I T E R A T U R E

1. Methods of Isolating Salmonella

Numerous attempts have been made to devise simpler, more efficient methods for isolating Salmonella, yet opinions differ on recipe, size of inoculum, when to subculture, incubation temperature, frequency of subculture, and on whether to use direct plating, enrichment, pre-enrichment or secondary enrichment methods.

CORRY 1969 and his co-workers investigated the complex interactions of cell damage, enrichment medium, temperature and duration of enrichment, and plating medium. From their studies they concluded that the most satisfactory combination of factors involving use of selective inhibitors appeared to be enrichment in tetrathionate broth incubated at 37° C followed by plating on brilliant-green phenol-red agar for 48 hr. They also reported that after attaining maximum numbers after 18 hr enrichment in selenite broth, Salmonella died off rapidly or, if the cells are damaged, they may fail to grow at all in this enrichment medium. That is why they did not recommend at all incubation at 43° C in either selenite or tetrathionate medium to isolate damaged Salmonella, except after pre-enrichment.

It was stated by ALFORD and KNIGHT 1969 that Enterobacteriaceae, occurring in dried foods and feeds, may carry metabolic lesions that impair the physiology of the cells to the extent that they will not proliferate in the customarily used selective media containing inhibitors in concentrations that are well tolerated by nonimpaired cells of the same species or serotype. Supporting this idea, NORTH 1961 suggested that non-selective pre-enrichment of Salmonella from dried albumin is useful because

the cells are presumably attenuated by prolonged storage in the dried state and, consequently, initially sensitive to selective agents such as selenite or tetrathionate.

MILLER and BANWART 1965 used various concentrations of brilliant-green and bile salts to study inhibition of *Salmonella* species, along with other bacteria capable of fermenting manitol. They found that as the concentrations of bile salts increased, the inhibitory effect of brilliant-green toward *Salmonella* decreased.

HOAG and ROGERS 1961 reported that *S. typhimurium* in mice could be isolated more frequently by tetrathionate enrichment medium used in conjunction with brilliant-green agar than on brilliant-green agar alone.

PALUMBO and ALFORD 1970 stated that when isolating *Salmonella* from sparsely infected material it is necessary to inoculate the material into enrichment media which allow the growth of *Salmonella* while restricting the undesirable Coliform and other gram-negative bacteria.

SHARMA and PACKER 1969 in their evaluation of culture media for the isolation of *Salmonella* from faeces, conducted a study on 300 faecal samples from a cow and a pig, each artificially contaminated with four *Salmonella* organisms, and reported that of the three enrichment broths they used in conjunction with the three selective media the maximum number of isolations they obtained were with brilliant-green MacConkey broth, followed by those obtained with tetrathionate broth and the least with selenite broth.

After comparing five liquid enrichment media (selenite F medium, tetrathionate broth, cacotheline broth, liquid desoxycholate-citrate medium and brilliant-green peptone water), SMITH 1952 reported that selenite broth and tetrathionate broth were greatly superior to the other enrich-

ment media. He added that selenite broth was preferable to tetrathionate for examining cow and chicken faeces, but that the reverse was true in the case of dog faeces.

GUINEE and KAMPELMACHER 1962 found that Müller-Kauffmann's medium and selenite brilliant-green broth detected equal numbers of *Salmonella* in infected porcine faeces and skin scrapings, though the number of positive findings was increased by using both media simultaneously.

HEIDRICH 1963 reported also that there was no appreciable difference between tetrathionate and selenite broths with regard to the isolation of *Salmonella* from faecal and organ samples.

KOOPMANN and JANSSEN 1972 examined faeces of 302 dogs and cats for *Salmonella* infection, from which they isolated 20 (6.58 %) *Salmonella* strains. They used the method of direct inoculation of 1 g of faeces into 10 ml of tetrathionate (Müller-Kauffmann) and selenite (Osborne and Stokes). Tetrathionate was incubated in temperatures of 37° C and 43° C for 48 hr, and selenite was incubated only at 37° C for 48 hr. This led to the result that tetrathionate incubated at 37° C proved to be the best with 75 % of the isolations, followed by the same enrichment medium incubated at 43° C with 45 % of the isolations, while selenite incubated at 37° C gave the least result with 25 % of the isolations.

McCOY 1962, working with samples of a sewage effluent, stated that the method of isolating *Salmonella* with selenite broth incubated at 43° C was disappointing because in comparison with other methods, the number of positives was significantly small. He also added that at 43° C tetrathionate broth was lethal to *Salmonella*, while among other workers, HARVEY and THOMSON 1953, GEORGALA and BOOTHROYD 1965, HARVEY and PRICE 1968, and ELLIOTT and

HEINIGER 1965 have appreciably used 43° C as an incubation temperature for selenite and tetrathionate enrichment broths. They reported also that many organisms which are able to grow and compete with *Salmonella* in inhibitory media at 37° C cannot do so at 43° C, whereas *Salmonella* do grow at this temperature.

WELLS et al. 1971 reported that an enrichment broth incubation temperature of 43° C is better than 37° C for isolating *Salmonella* from milk.

BÄNFFER 1971, in his work about the comparison of the isolation of *Salmonella* from human faeces by enrichment at 37° C and at 43° C, stated that incubation of an enrichment broth at 43° C seems to be appropriate for the isolation of *Salmonella* from scanty infected faeces.

MORRIS and DUNN 1970 compared the effect of different incubation temperatures on the isolation of *Salmonella* from pork sausage with and without added Tergitol. They pointed out that in the absence of added Tergitol, the 43° C incubation temperature was superior to incubation at 37° C. However, very little difference was noted between the 24-hr and the 48-hr incubation period, whereas TAYLOR and SILLIKER 1961 reported that they found more positive cases from samples which had been incubated in selenite broth for 48-hr than for 24-hr.

Discussing the effect of incubation time of selenite and tetrathionate media on the isolation of *Salmonella* from lightly infected faeces, SMITH 1959 reported that the optimum time of incubation at 37° C in the case of tetrathionate was between 24 hr and 30 hr. The efficiency of this medium then decreased until 48 hr. In the case of selenite medium the maximum number of isolations obtained was at 30 hr, but in contrast to tetrathionate medium, there was no falling off of efficiency at 36 hr and 48 hr.

Taking into consideration that bacteria complicate the work of the isolation of Salmonella from faeces, BALL 1951 stated that although on subculturing from the enrichment medium the colonies of Proteus did not spread over SS plates they were still definite contaminants. Being non-lactose fermenters they were frequently picked for transfer to the slants of Kligler's iron agar medium.

ADLER et al. 1951 also found in the course of attempted isolation of Salmonella a large number (estimated 75 %) of the colonies picked to Kligler's iron agar turned out to be members of the genus Proteus. To avoid such problems, STUART and PIVNICK 1965 presented a method for isolating Salmonella from naturally infected faeces by means of selective migration through semi-solid enrichment media in modified "U" tubes. They found out that for the recovery of Salmonella the selective motility system techniques were equal or superior to the standard procedures employed in routine diagnostic laboratories.

2. The Incidence of Salmonellosis in Dogs

All Salmonella types are potential pathogens for animal and man or both and each host may be a source of infection to another member of the same or a different species. It is significant, therefore, to observe the frequency of Salmonella isolations from dogs.

MATSUMOTO 1966, in his work about studies on Salmonella in dogs, reported that from 1,341 mesenteric lymph nodes of apparently healthy stray dogs in Osaka, strains of Salmonella with 16 serotypes were isolated from 188 (14 %) dogs, with the commonest serotype S. typhimurium (33.8 %) followed by S. potsdam (15.8 %), S. thompson (14.3 %) and S. give (13.3 %).

In the Netherlands, GOUDSWAARD 1969 found 34 (11.3 %)

positive cases with 12 serotypes of Salmonella from 300 specimens of the bowel contents of dogs which were referred to the Institute of Veterinary Pathology for post-mortem examination during the period from July 1, 1967 to July 1, 1968. After this result, the widely held view based on Swedish, Italian and English studies carried out ten years ago, that Salmonella infection in dogs was not very common in Europe, is no longer acceptable to him.

GALTON et al. 1952 reported that, from a total of 1,626 normal family dogs examined in Florida, 244 (15 %) were positive for Salmonella infection.

ADLER et al. 1951 reported that Salmonella were detected in 39 (13.2 %) of 294 apparently healthy dogs at the quarantine station in Hawaii.

SCHMIDT 1970 cited four outbreaks of salmonellosis in dogs in pet shops in West Berlin. From these dogs one case of *S. typhimurium*, 2 cases of *S. infantis* and one case of *S. panama* were isolated.

Of 442 dogs examined by KHAN 1970 in the Sudan for the presence of Salmonella 104 (23.5 %) were found to be infected. One hundred and twenty-nine strains were recovered and were classified into 46 serotypes belonging to 12 "O" groups.

In 1960/61 MESSOW and HENSEL 1962 examined organs of 135 dogs which were referred to the Institute of Veterinary Pathology in Hannover for post-mortem examination and reported 4 (3 %) outbreaks of Salmonella from which two different serotypes were isolated. In three cases *S. typhimurium* was identified and in one case *S. enteritidis*.

QUEISSER 1970 reported three outbreaks of salmonellosis - *S. derby*, *S. taksony* and *S. heidelberg* - in clinically healthy service dogs of the Bundeswehr in the Federal

Republic of Germany. The result was obtained during systematic performance of bacteriological examination of 314 dogs' faecal samples.

JUNGERMAN and GRUMBLES 1960, in Texas, examined 100 dogs over two years of age. Only dogs in good health and with no history of illness within 6 months or diarrhoea within 12 months were used. With few exceptions all were household pets living in a home environment. From faecal specimens obtained by rectal swabbing, 9 yielded *Salmonella* organisms of the 100 dogs tested. Two dogs were infected with 2 species each. All isolations were typed serologically and 9 different species were found. Two dogs were retested 6 weeks after a positive initial test and three dogs 6 months after a positive initial test; all were found to be negative. The authors stated that their study was undertaken with regard to further illumination of the possibility of healthy dogs serving as sources of *Salmonella* infection for humans.

FULTON et al. 1956 made a test on 9 dog faecal samples, from which 7 were positive for *Salmonella* infection divided into 5 different serotypes. They stated that all types were known to be pathogenic to man.

GALTON et al. 1955 found 17 *Salmonella* serotypes from commercial dog meats, and reported that all 17 were also isolated from dogs in Florida; 15 of these were isolated from human beings in the same place.

MANN 1969 isolated three *S. newport* and one *S. schwarzengrund* from the faeces of 4 dogs out of 200 dogs he examined for *Salmonella* infection. Although he found relatively few dogs infected with *Salmonella*, he drew attention to the fact that one carrier animal may cause widespread dissemination of salmonellosis in a given area. This statement assumes greater importance in view of the

fact that Salmonella is known to survive in the soil for a long period of time.

KINTNER 1949 described that a number of dogs carry Salmonella organisms in their digestive tracts without suffering any discernible ill effects, and he added that the incidence of Salmonella infection was much higher in dogs suffering from distemper than in the other dogs he examined.

BALL 1951 presented a report of 300 dogs he examined for Salmonella infection; 17 (5.66 %), four of which were young animals, were excreting Salmonella organisms.

BUTLER and HERD 1965 examined, in Alaska, a total of 173 faecal samples of dogs which they divided into two groups: sled dogs (kept in kennels) and family pets. In the first group, which consisted of 75 animals, no Salmonella was isolated; in the second group (family pets), which consisted of 98 animals, 16 (16.2 %) were positive for Salmonella and these animals had been infected throughout the year.

CARAWAY et al. 1959 made several examinations to determine the cause of an enteric disease in sentry dogs at Chennault Air Force Base in New Orleans. In their first survey from faecal specimens of 23 dogs, 18 yielded positive Salmonella cultures including 12 different serotypes, with some specimens containing two or more types. The second survey, four months later, revealed Salmonella in 23 dogs. Ten serotypes were found, 4 of which had been isolated in the first survey, but in no instance was the same type isolated from the same dog in both surveys. From this they concluded that the result supports the theory that Salmonella infections in dogs are of short duration and exposures are frequent.

3. The Role of the Dog in the Epidemiology of Human Salmonellosis

KAUFFMANN and HENNINGSEN 1939 reported an outbreak of gastroenteritis involving six members of a family of seven and the family dog. *S. glostrup* was isolated from the faeces of all members of the family and from the blood of the infected dog.

DAY et al. 1963 stated that examination of a single faecal specimen may lead to an erroneous conclusion when animals are infected with *Salmonella* organisms, since the shedding state of naturally exposed dogs may be quite sporadic. Nine dogs were given feed contaminated with 4 or more *Salmonella* serotypes for periods of 16 - 50 days. Seven of the 14 serotypes being fed were recovered from the dogs along with 2 additional serotypes which were not demonstrated in the dog feed. In another trial, 13 dogs were fed large numbers of *S. worthington* or *S. infantis* in a single dose. No clinical signs of illness were observed but a prolonged and sporadic shedder state was produced. It appeared that dogs being fed a contaminated feed might constitute a human health hazard, since they might shed *Salmonella* organisms sporadically without clinical signs of illness.

CASPERSEN 1937 reported a case in which an infected dog was the source of a paratyphoid outbreak involving six human beings. WOLFF et al. 1948, discussing *Salmonella* from dogs and the possible relationship to salmonellosis in man, considered dogs to be the potential source of *Salmonella* infection for man.

Van der SCHAAF 1961 stated in his work "Salmonellosis in Carnivorous Domestic Animals and Fur Animals" that the rise in *Salmonella* contamination of meats resulted to a certain extent in a corresponding increase of salmonel-

losis in man and carnivorous domestic animals.

BALL 1951, while discussing Salmonella in dogs and cats of the Los Angeles, Honolulu and Bermuda areas, explained that dogs and cats which were discharging Salmonella were the potential source of infection for themselves, other animals and man.

GIBSON 1961, discussing salmonellosis in calves, reported that from 8 farm dogs there were 6 positive cases. S. typhimurium was isolated from two separate cases and S. dublin from a third on the farms where these serotypes were present in calves. This investigation led him to the conclusion that dogs might be of some importance in the transmission of infection from calves to man and especially to young children.

THOMPSON and WRIGHT 1969 explained that the risk to human health when exposed to cases of salmonellosis, especially involving family pets in lethal infections, is always present.

THAL et al. 1957 contended that Salmonella infection could involve several of the animals on a farm and that as far as obvious pathological lesions were concerned, apparently healthy animals could be carriers of the organisms.

TAYLOR 1967 stated that any factor which enabled a type of Salmonella to become entrenched in a domestic species would result in an increase in human infection due to this type, because in general the common Salmonella types found in man are those common in domestic animals.

GALTON et al. 1955 reported that Salmonella types which were isolated frequently from dogs were also encountered frequently in man. BUXTON 1957 related that although there was a potential danger to human health from handling in-

fectured domestic pets, the risks should not be over-emphasized and the greatest hazard could probably be associated with the nursing of clinically infected animals in the home. He added that in these circumstances the owner should be made aware of the importance of cleanliness and disinfection when handling such animals. Whatever the danger might be, it could be reduced by paying careful attention to personal hygiene and by insuring that food-stuffs fed to domestic pets were free from Salmonella infection.

SMITH 1959 stated that the part played by dogs and cats in spreading Salmonella infection to man was also not fully understood. However, their relatively high rate of infection with Salmonella and the fact that they had been shown to have been responsible for some cases of human infection, indicated that they should be considered as possible sources for human salmonellosis.

III. MATERIAL AND METHODS

1. Material Examined

The natural habitat of Salmonella is mostly the gastro-intestinal tract of animals and human hosts. Salmonellosis is, therefore, primarily an excremental disease transmitted by the faecal-oral route. For this reason, only faecal specimens were obtained as material to be examined in this study.

Faecal specimens were collected from three different sources:

Samples were obtained from 71 dogs which were referred to the Institute of Veterinary Pathology, Free University Berlin, for post-mortem examination.

Samples were collected from 75 dogs which had defecated their faeces on different streets of West Berlin.

Samples were obtained from 84 dogs which were kept in pet shops, in West Berlin, ready for sale.

2. Testing Media

In this study one resuscitation medium, two selective enrichment broths and two solid plating media were used.

Resuscitation medium

Tryptone Soya Broth (TSB) is used as a resuscitation medium.

Its composition consists of the following:

Pancreatic digest of casein (Oxoid L 42)	17.0 g
Papaic digest of soybean (Oxoid L 44)	3.0 g
Sodium chloride	5.0 g
Dibasic potassium phosphate	2.5 g
Dextrose	2.5 g
Distilled water	1,000 ml

Selective enrichment media

Selenite brilliant-green manitol enrichment medium acc. to Osborne and Stokes.

Composition (g per litre):

Special peptone	5.0
Extract of yeast	5.0
D (+)-manitol	5.0
Sodium selenite	4.0
Brilliant green	0.005
Potassium dihydrogen phosphate	3.4
di-Potassium hydrogen phosphate	4.35
(pH of the made-up medium at 35° C: 6.8 - 7.2)	

Potassium tetrathionate-brilliant green-bile-enrichment broth (Merck Art. No. 5172).

Composition (g per litre):

Special peptone	8.6
Bile desiccated	8.0
Sodium chloride	6.4
Calcium carbonate	20.0
Potassium tetrathionate	20.0
Brilliant green	0.07
(pH of the made-up medium at 37° C: 6.9 - 7.1)	

Selective plating media

Litmus lactose agar acc. Drigalski

Composition (g per litre):

Special peptone	6.9
D (+)-lactose	15.0
Litmus	1.2
Sodium chloride	5.1
Agar agar	13.0
(pH of the prepared culture medium at 37° C: 7.2 - 7.6)	

Brilliant-green phenol-red lactose agar acc. to Kauffmann.

Composition (g per litre):

Special peptone	6.9
Lactose	15.0
Phenol red	0.04
Brilliant green	0.005
Sodium chloride	5.1
Agar agar	13.0

(pH of the made-up medium at 37° C: 6.4 - 6.6)

3. Methods Applied

Faecal samples numbering 230 were collected from the same number of dogs of three different sources as explained above.

With the aim of achieving the comparatively best method of isolating Salmonella from faeces, sixteen enrichment methods and two plating media were used in parallel, which means that thirty-two different trials had been compared. Eight of these enrichment methods were used with resuscitation and the other eight were used without resuscitation.

Resuscitation Method

Tryptone Soya Broth (Soybean-casein digest) which was prepared in the following way was used as a resuscitation medium. Thirty grams of the powder medium was well mixed with 1 litre of distilled water and was distributed into 200-ml Erlenmeyer flasks with 50 ml of the medium in each flask. Then it was sterilized by autoclaving at 121° C for 15 minutes. After the medium was cooled to room temperature, 3 g of each faecal specimen were dropped into each flask containing the medium and was stirred until it was well mixed.

In this case the resulting fluid layers were not much

deeper than 1 cm, which means that it was shallow enough for good aeration. Incubation was carried out at room temperature (20 to 25° C) for 2 to 3 hr. After this resuscitation period 3 ml of the fluid-mixture from each specimen (as it is shown in diagram 1a) was transferred to each of the four standard-culture-tubes, two containing 10 ml of tetrathionate each and two containing the same amount of selenite.

Two tubes of the inoculated selective broths (one selenite and one tetrathionate) were incubated at 37° C and the other two at 43° C.

Non-resuscitation Method

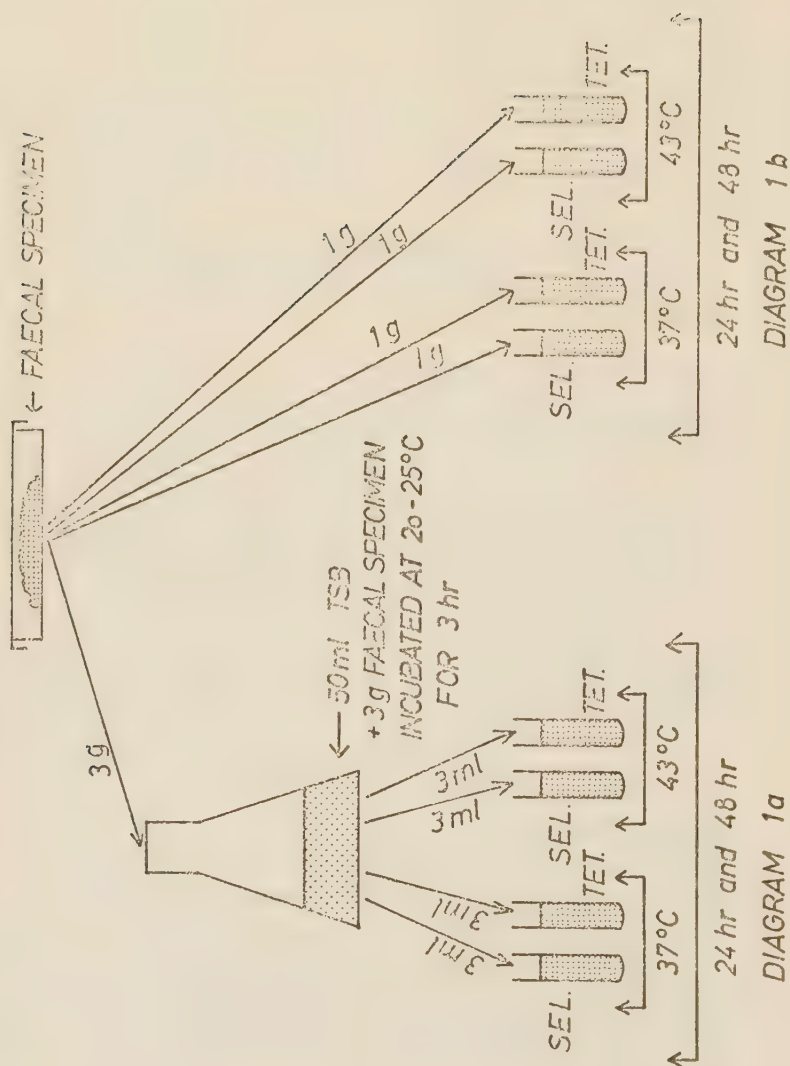
From the remaining faecal specimens, about 1 g of the material was dropped directly into each of the four enrichment broth tubes which contained tetrathionate and selenite (as it is shown in diagram 1b). As it was done with the first method, one pair of selenite and one pair of tetrathionate tubes were incubated at temperatures of 37° C and 43° C respectively.

When the incubation process of the selective enrichment media was performed in both methods, one pair of the enrichment cultures was incubated at 37° C and the other part at 43° C. The samples were, therefore, paired.

Plating Process

After exactly 24-hr incubation, 0.02 ml of the culture broth incubated at 37° C was streaked to a half-plate of brilliant-green phenol-red lactose agar (BPRA), and the same amount of the specimen to a half-plate of litmus lactose agar (LLA). The same process was performed with the culture broths incubated at 43° C which were then streaked on the remaining halves of the same plates. The streaked plates were incubated at 37° C for 24 hr. The

ENRICHMENT METHODS



enrichment cultures were again incubated for further examination, which means 48 hr altogether from the time of inoculation.

Isolation and Identification of Salmonella Colonies from Plate Media

Usually, twelve to sixteen suspicious colonies per specimen were picked and subcultured into a new plate. Characteristic Salmonella colonies picked from the medium may be agglutinated directly without subculturing and preliminary incubation when the specimen likely to contain only a single serotype is to be examined. But the faecal specimen from a dog may contain Salmonella of more than one serotype, and direct agglutination of colonies picked in succession from the first plate may lead to discordant results. In addition, it is convenient to have for slide agglutination more material than is contained in a single colony. If a plate appeared suspicious but no isolated colonies were present, the suspicious growth was transferred directly to a fresh plate and streaked for isolation.

Colonies of Salmonella characteristics were subcultured to Kligler's iron agar. Those which showed with Kligler's iron agar a reaction characteristic of one of the Salmonella groups (lactose -, glucose + and H_2S $\pm$) were then first screened serologically with a polyvalent Salmonella serum in a slide test. If a positive reaction was obtained, the culture was typed serologically according to the Kauffmann-White scheme. If the culture was negative in the slide agglutination test, after gram test, it was inoculated into urease medium, tryptophane broth for the production of indol. It was also tested for Voges-Proskauer, methyl-red, lysin, arginin and ornithin reactions. Cultures which showed biochemical reactions characteristic of Salmonella were then sent for further examination to

Prof. Dr. Bulling and Dr. Pietzsch, Salmonella Typing Center, Bundesgesundheitsamt in West Berlin.

The outcome of each sample was then recorded according to the model of Table 1.

TABLE 1. MODEL OF THE RECORD USED FOR EACH SAMPLE

Sampling Date.....

Record No.

Enrichment Media	24 hr 37°C	
	BPRA	LLA
Sel.		
Tet.		
Sel. (TSB)		
Tet. (TSB)		

Enrichment Media	48 hr 37°C	
	BPRA	LLA
Sel.		
Tet.		
Sel. (TSB)		
Tet. (TSB)		

Enrichment Media	24 hr 43°C	
	BPRA	LLA
Sel.		
Tet.		
Sel. (TSB)		
Tet. (TSB)		

Enrichment Media	48 hr 43°C	
	BPRA	LLA
Sel.		
Tet.		
Sel. (TSB)		
Tet. (TSB)		

Type of Salmonella Isolated.....

Key: 24 and 48 hr = time taken for incubation of enrichment media
 37° C and 43° C = incubation temperatures
 Sel. = selenite
 Tet. = tetrathionate
 TSB = tryptone soya broth (resuscitation medium)
 BPRA = brilliant-green phenol-red lactose agar
 LLA = litmus lactose agar (acc. Drigalski)

IV. RESULTS

1. The Incidence of Salmonella Infection in Dogs in West Berlin

Table 2 shows the result of 230 faecal samples from the same number of dogs examined for Salmonella infection in West Berlin.

TABLE 2. NUMBER OF SALMONELLA ISOLATIONS FROM 230 DOGS' FAECAL SAMPLES EXAMINED

No. of samples examined	No. of positive samples	No. of Salmonella strains isolated	No. of Salmonella serotypes isolated
230	59	66	15

From 230 samples, 59 specimens were found to be positive from which 66 strains of Salmonella were isolated and these strains comprised 15 serotypes. In this case there was an incidence of multiple infection where each of 7 dogs yielded two serotypes, but there was no incidence of isolating three or more serotypes from a single faecal specimen.

The frequency with which Salmonella organisms were isolated from the faeces of dogs from the three different origins is shown in Table 3.

TABLE 3. THE INCIDENCE OF SALMONELLA INFECTION
IN DOGS FROM THREE DIFFERENT SOURCES

Origin	Number sampled	Number infected	% infected
Faeces collected from Pathology Institute	71	13	18.3
Faeces collected from streets	75	11	14.6
Faeces collected from pet shops	84	35	41.7
Total	230	59	25.7

From the intestinal content of 71 dogs which were referred to the Institute of Veterinary Pathology for post-mortem examinations, 13 (18.3 %) with 7 types of Salmonella were isolated (60 % of the infected dogs were under 3 months of age).

From the 75 faecal samples of dogs which were collected from different streets of West Berlin, 11 (14.6 %) positive cases with 7 types of Salmonella were found (in this case, recording the age of the dogs was not possible).

From 84 dog faecal samples which were obtained from four different pet shops, the highest number, 35 (41.7 %) positive cases with 8 types of Salmonella were isolated (80 % of the infected dogs were under 3 months of age).

It was only in the last case that an incidence of multiple infection was found, in which each of 7 dogs yielded two types of Salmonella.

The incidence of Salmonella infection in four pet shops (A, B, C and D) which are located in four different

districts in West Berlin is shown in Table 4.

TABLE 4. THE INCIDENCE OF SALMONELLA INFECTION
IN DOGS IN FOUR PET SHOPS

Origin of dogs	Number sampled	Number infected	% infected
Pet Shop A	24	10	41.7
Pet Shop B	34	12	35.3
Pet Shop C	16	4	25.0
Pet Shop D	10	9	90.0
Total	84	35	41.7

According to the information given by the owners, all the dogs were imported from four or more European countries. In comparison with the samples collected from the streets and from the Institute of Veterinary Pathology, the infection rate of the dogs from the four pet shops is considerably higher. The highest incidence and the most severe forms were observed in pet shop D, where out of 10 dogs, 9 (90 %) were infected with only one type of *Salmonella*, i.e. *S. typhimurium*. Even in the other pet shops, where incidences of multiple infections occurred, *S. typhimurium* was found to be the predominant type. In 4 of the infected young dogs from pet shop D and 6 from the other pet shops, clinical manifestations of infection (which could be due to the salmonellosis) were observed characterized by dullness, anorexia, thinness, as well as fluid and haemorrhagic stools.

Statistical Data

The significances of the difference of *Salmonella* infection rate (with a given level of significance of 1 %):

a) in dogs from pet shops in relation to the dogs which

were referred to the Institute of Veterinary Pathology for post-mortem examination was $9.82 > 6.62$,

- b) in dogs from pet shops in relation to the dogs which defecated their faeces on the different streets of West Berlin and dogs from the Institute of Veterinary Pathology was $18.08 > 6.62$,

are statistically analysed according to the "fourfold table" scheme.

The "fourfold table" scheme was also applied to show the significance of the difference of Salmonella infection rate in dogs from the different pet shops:

- a) pet shop D (where the highest number of positive cases were found) in relation to pet shop C (where the lowest number of positive cases were found) = $10.4 > 6.62$,
- b) pet shop D in relation to pet shop A (where the second highest positive cases were found) = $6.69 > 6.62$.

These significances of difference show the heterogeneity of the Salmonella infection rate in dogs in different parts of West Berlin.

2. Salmonella Strains and Serotypes Isolated
from the Examined Dogs

The distribution of Salmonella types isolated from dogs' faeces of different origins is shown in Table 5.

TABLE 5. SEROTYPES OF SALMONELLA ISOLATED
FROM DOGS' FAECES

Serotype	Pathology Dogs	Street Dogs	Pet Shop Dogs	Total
S. agona		1		1
S. anatum	1			1
S. brandenburg			5	5
S. bredeney			2	2
S. derby	1			1
S. enteritidis	1	1	3	5
S. give		1		1
S. heidelberg		2	1	3
S. infantis	1	1		2
S. manhattan			3	3
S. newington	1			1
S. oranienburg			1	1
S. panama		1	4	5
S. saint-paul	1			1
S. typhimurium	7	4	23	34
Total Strains	13	11	42	66

Of the 66 Salmonella strains isolated, 15 different serotypes were identified. From these the most prevalent serotypes were S. typhimurium (51.5 %), S. panama (7.5 %), S. brandenburg (7.5 %) and S. enteritidis (7.5 %).

S. anatum, S. derby, S. newington and S. saint-paul were isolated only from dogs which were referred to the Insti-

tute of Veterinary Pathology for post-mortem examinations. Two of these types were isolated also from the organs of the same dogs, i.e. *S. derby* was isolated from the intestines, mesenteric lymph nodes, and kidneys; *S. anatum* was isolated from the lymph nodes only.

S. agona and *S. give* were isolated only from dogs' faecal samples which were collected from the streets. *S. brandenburg*, *S. bredeney*, *S. manhattan* and *S. oranienburg* were recovered from the dogs' faecal samples which were obtained from the pet shops. The other five types, i.e. *S. typhimurium*, *S. enteritidis*, *S. heidelberg*, *S. panama* and *S. infantis* were isolated from two or all three sources.

3. Factors Influencing the Isolation of Salmonella from Naturally Infected Faeces of Dogs

A series of comparative influence of pre-enrichment medium, selected enrichment broths, duration of enrichment, incubation temperatures and selective plating media for the isolation of *Salmonella* from naturally infected faeces of dogs is shown in Tables 6a, 6b and 6c.

Table 6a shows a comparison of the 24- and 48-hr duration of enrichment of faecal specimens in two selective broths incubated at 37° C with resuscitation and non-resuscitation methods.

TABLE 6a. NUMBER OF SALMONELLA ISOLATIONS MADE AT 37° C
WITH AND WITHOUT RESUSCITATION METHODS

Methods	Enrichment Media	24 hr 37°C		48 hr 37°C	
		BPRA	LLA	BPRA	LLA
Non-Resuscitation	Tet.	29	26	43	38
	Sel.	13	11	27	19
Resuscitation (with TSB)	Tet.	28	23	48	46
	Sel.	10	9	29	25
Total of Isolations		80	69	147	128

Tet. = tetrathionate

Sel. = selenite

TSB = tryptone soya broth

BPRA = brilliant-green phenol-red lactose agar

LLA = litmus lactose agar

Table 6b shows a comparison of the 24- and 48-hr duration of enrichment of the faecal specimens inoculated in two selective enrichment broths incubated at 43° C with resuscitation and non-resuscitation methods.

TABLE 6b. NUMBER OF SALMONELLA ISOLATIONS MADE AT 43° C
WITH AND WITHOUT RESUSCITATION METHODS

Methods	Enrichment Media	24 hr 43°C		48 hr 43°C	
		BPRA	LLA	BPRA	LLA
Non-Resuscitation	Tet.	41	39	34	32
	Sel.	14	15	9	7
Resuscitation (with TSB)	Tet.	56	51	46	45
	Sel.	19	17	17	13
Total of Isolations		130	122	106	97

a) Comparison of the Sensitivity of Plating Media
for the Isolation of Salmonella

Regardless of the variations in the fluid enrichment procedures made, Tables 6a and 6b show that plating medium BPRA with 463 isolations was superior to LLA plating medium from which 416 isolations were obtained.

b) Comparative Efficiency of Tetrathionate Broth and
Selenite Broth for the Isolation of Salmonella
from Faeces of Dogs

The efficiency of the two enrichment broths with resuscitated and non-resuscitated specimens at 24- and 48-hr duration of enrichment and 37° C and 43° C incubation temperatures used in conjunction with BPRA and LLA plating media is shown in Table 6c.

The number of isolations from both selective media in both methods under various conditions were compared. From 397 possible isolations made with non-resuscitation method, 282 (71 %) were obtained from tetrathionate broth and 115 (29 %) from selenite broth, whereas with resuscitation method from 482 possible isolations made, 343 (71 %) were obtained from tetrathionate and 139 (29 %) from selenite broth.

Even though, as to the number of isolations made, the difference between tetrathionate and selenite broths in resuscitation and non-resuscitation methods is great, the proportional difference between the two enrichment broths is more or less the same in both methods.

c) The Influence of the Incubation Temperature on the
Isolation of Salmonella from Faeces of Dogs

As it is shown in Tables 6a, 6b and 6c, the two comparable incubation temperatures for the two enrichment broths

TABLE 6c. COMPARISON OF THE PRODUCTIVITY OF RESUSCITATION AND NON-RESUSCITATION METHODS FOR THE ISOLATION OF SALMONELLA FROM DOGS' FAECES

Methods of isolation	Enrichment Media (incubated at 37° C and 43° C)	Recovery after primary enrichment (incubation time 24 hr)		Recovery after secondary enrichment (incubation time 48 hr)		Total number of isolations
		BPRA	LLA	BPRA	LLA	
Non-Resuscitation	Tet. 37° C	29	26	43	38	397
	Tet. 43° C	41	39	34	32	
	Sel. 37° C	13	11	27	19	
	Sel. 43° C	15	15	9	7	
Resuscitation (with TSB)	Tet. 37° C	28	23	48	46	482
	Tet. 43° C	56	51	46	45	
	Sel. 37° C	10	9	29	25	
	Sel. 43° C	19	17	17	13	

Tet. = tetrathionate

Sel. = selenite

TSB = tryptone soya broth

BPRA = brilliant-green phenol-red lactose agar

LLA = litmus lactose agar

used in this study were 37° C and 43° C.

From the 397 isolations made with non-resuscitation method, 206 (52 %) were obtained from enrichment media incubated at 37° C and 191 (48 %) at 43° C. But in the resuscitation method, the case was the reverse: from 482 possible isolations made, 218 (45 %) were obtained from enrichment media incubated at 37° C and 264 (55 %) at 43° C.

d) The Influence of the Duration of Enrichment of Dogs' Faecal Samples in Selective Enrichment Media on the Isolation of Salmonella

If the duration of enrichment is taken as a separate entity - although the duration of enrichment is dependent on the incubation temperature and the selectivity of the media - with non-resuscitation method from a total of 397 isolations, 188 (47 %) were found after 24-hr incubation and 209 (53 %) isolations were made after 48-hr incubation (as it is shown in Table 6c). With the resuscitation method from a total number of 482 isolations, 213 (44 %) were found after 24-hr incubation and 269 (56 %) isolations were made after 48-hr incubation. This means that the difference between the 24-hr and 48-hr enrichment is more significant with the resuscitation method than with the non-resuscitation method.

The most generally satisfactory combination of factors involving use of selective inhibitors for the isolation of Salmonella from faecal samples of dogs appeared to be enrichment in tetrathionate broth incubated, after resuscitation, at 43° C for 24 hr followed by plating on brilliant-green phenol-red lactose agar.

e) The Comparative Efficiency of the Resuscitation
and Non-Resuscitation Methods

Table 7 shows the effect of resuscitation method for the isolation of Salmonella from naturally infected faeces of dogs.

TABLE 7. COMPARISON OF THE PRODUCTIVITY OF RESUSCITATION
AND NON-RESUSCITATION METHODS FOR THE ISOLATION
OF SALMONELLA FROM FAECES OF DOGS

Total samples examined	Positive by all methods	Positive by resus- citation method	Positive by non- resusci- tation method	Positive by resuscitation method but ne- gative by non- resuscitation method
230	59	59	42	17

Without any resuscitation treatment, only 42 out of 59 positive samples would have been found, which means that 17 positive samples for Salmonella infection (which is slightly under 30 % of the positive cases) would have been missed by immediate inoculation of the faecal specimens into selective enrichment media.

4. Statistical Analysis

The effect of the different factorial combinations (Tables 6a, 6b and 6c) of the following variables were statistically analysed:

Enrichment method (A)	= resuscitation, non-resuscitation
Time of subculture (B)	= 24 hr, 48 hr
Temperature of incubation (C)	= 37° C, 43° C
Plating media (D)	= BPRA, LLA
Enrichment media (E)	= Tet., Sel.

The statistical analysis of the experimental result was done according to 2^5 factorial design (WEISS 1969). It has been established, in this study, that from two sets of five factors, $2^5 = 32$ different combinations were possible. A single- and multi-factorial analysis of variance for all possible combinations, to compare the effect of the individual factors on recovery and their interactions, were performed. Arc-sin-transformation was then carried out in order to stabilize the variances of the observed attributes (WINER 1970). As a result, the analysis of variance of the main effect of change in factors A, B, D and E was found to be significant (with significance level of 1 %); as well the bi-factorial interaction effects of AB, AC, BC and CE (as shown in Table 8) were also found to be significant. The effect of the interaction of the remaining 6 bi-factorial and the other multi-factorial possible combinations was, however, found to be a matter of chance.

TABLE 8. STATISTICAL ANALYSIS OF DATA PRESENTED
IN TABLES 6a, 6b AND 6c

A X C	24 hr	48 hr
Res.	213	269
Non-Res.	188	209

A X C	37°C	43°C
Res.	218	264
Non-Res.	206	191

B X C	37°C	43°C
24 hr	149	252
48 hr	275	203

C X E	Tet.	Sel.
37°C	281	143
43°C	344	111

The statistical interactions observed emphasize that in choosing the enrichment method, enrichment medium, incubation temperature and time of subculture each of these factors has to be considered in relation to the others.

V. DISCUSSION

1. The Efficiency of Methods for Isolating Salmonella from Faecal Samples of Dogs

The most interesting observation in this study was the definite superiority of the resuscitation method (with 59 positive specimens) over the non-resuscitation method (with 43 positive specimens) for recovering Salmonella from the naturally infected faecal specimens of dogs. This means positive samples would have been reported as negative in 16 of 59 instances if only the non-resuscitation method had been applied.

MOSSEL and RATTO 1970 stated that cells normally resistant to selective agents become sensitive to them when exposed to sublethal heating or drying. The result of this study, where 30 % of the positive samples have been found only through the resuscitation method, also supports the findings of these authors that the resuscitation method increased the positive samples of dried foods for the isolation of Enterobacteriaceae by 40 %.

The difference in the efficiency of resuscitation and non-resuscitation methods seems to indicate that perhaps tetrathionate and selenite broths exercise some inhibitory effect on Salmonella when the faeces are minimally contaminated or the cells are one way or the other physiologically injured. The presumed function of pre-enrichment treatment is to allow the injured cells a chance to begin active growth in a non-inhibitory medium. If the major inhibitory effect of selenite and tetrathionate on injured Salmonella disappears by the time the cells have undergone one or two multiplications, then the resuscitatorial treatment should minimize the effects of the inhibitory media on Salmonella without affecting their inhibition on other coliform bacteria. Thus the optimal duration of exposure to the resus-

citation technique used for dogs' faeces appeared to be 2 to 3 hr. Apparently a longer restoration treatment has also led to partial overcrowding of the medium by other bacteria since there was little inhibition of the coliform bacteria once they were growing logarithmically.

HEIDRICH 1963 reported that there was no appreciable difference between tetrathionate and selenite with regard to the isolation of *Salmonella* from faecal and organ samples; however, the researcher found a big difference of efficiency between these two broths for the isolation of *Salmonella* from naturally infected dog faecal samples. In both methods tetrathionate was found to be superior to selenite broth:

- a) more specimens were shown to be positive by the use of tetrathionate than selenite,
- b) non-lactose fermenting colonies which were not *Salmonella* appeared more frequently after selenite enrichment rather than tetrathionate.

This fact also confirms the findings reported by SMITH 1952 and KOOPMANN and JANSSEN 1972 that tetrathionate broth gave the maximal number of isolations of *Salmonella* than selenite from dog faecal samples.

TAYLOR and SILLIKER 1961 considered tetrathionate to be more inhibitory than selenite, and McCOY 1962 reported that tetrathionate was lethal to *Salmonella* at incubation temperature of 43° C. Nevertheless, in this study 43° C has been more successfully used with tetrathionate than with selenite broth. The observations that 48-hr incubation time at 37° C was better than 24-hr for tetrathionate medium with non-resuscitated specimens, appear to conflict with the observations of SMITH 1952 and SHARMA and PACKER 1969, who noticed a decrease in the efficiency of tetrathionate beyond 30 hr of incubation. However, this may have been because the faecal specimens

they examined were artificially lightly infected samples, at the same time the formula for tetrathionate broth used by them differs slightly from that used in this study. There was another feature of the tetrathionate enrichment incubated at 43° C. The solid media inoculated from this enrichment broth displayed a greater pureness. The researcher had no difficulty in selecting *Salmonella* colonies for the slide agglutination and the percentage of the suspected colonies that appeared to be *Salmonella* was larger than after enrichment at 37° C.

A comparison was also made on the effect of the two incubation temperatures on the isolation of *Salmonella* from dog faecal samples with the resuscitation method. The result of the 24-hr enrichment indicated that the 43° C incubation temperature was superior to incubation at 37° C. The higher temperature suppressed the competing gram-negative bacteria and permitted *Salmonella* to grow in relatively pure culture, thus providing an advantage for isolating and identifying the organisms. In the lower incubation temperature, however, the competing bacteria tended to obscure the *Salmonella* colonies and rendered the examination more time consuming.

In spite of the fact that the 43° C incubation temperature for tetrathionate broth appeared to have good value in retarding the growth of other non-lactose fermenting bacteria without inhibiting *Salmonella*, with selenite broth incubated at the same temperature, however, the most troublesome problem in processing the naturally infected faecal cultures of dogs was the overgrowth by *Proteus*. This shows that obviously neither the high temperature alone nor the tetrathionate broth alone were responsible for the selectivity of the bacterial growth, but that both factors contributed towards the purity of the culture.

The analysis of data on the efficiency of the two selective plating media in relation to the two enrichment broths revealed maximal isolation on brilliant-green phenol-red lactose agar when compared to litmus lactose agar. The statistical interactions observed here emphasize that in choosing the enrichment broth, incubation temperature, duration of enrichment and plating medium, each of these factors has to be considered in relation to the other. Thus, taking into account these various and complex interactions, it appears from the data that the most satisfactory combinations for the isolation of *Salmonella* from naturally infected faecal samples of dogs were the resuscitated cultures enriched in tetrathionate broth incubated at 43° C for 24 hr followed by plating on brilliant-green phenol-red lactose agar.

2. The Incidence of Salmonella Infection in Dogs

The incidence of salmonellosis in dogs as reported by different workers varies considerably. BUXTON 1957, for example, reported that the *Salmonella* infection rate in dogs in Great Britain was only 1 %. GOUDSWAARD 1969 found 11.3 % positive cases in dogs in the Netherlands. CARAWAY et al. 1959 in New Orleans found a 78 % infection rate in 23 dogs examined. BUTLER and HERD 1965 reported of a 16.2 % *Salmonella* infection rate in dogs in Alaska. GALTON et al. 1952 reported that from a total of 1,626 normal family dogs examined in Florida, USA, 244 (15 %) were found to be positive for *Salmonella* infection. ADLER et al. 1951 reported that 13.2 % of the dogs at the quarantine station in Hawaii were infected with *Salmonella*. MATSUMOTO 1966 reported that 14 % of the dogs in Osaka, Japan, were found to be infected with *Salmonella*.

In Africa, the limited work carried out has shown that the incidence of *Salmonella* in dogs may also vary widely.

In Ghana, ZWART 1962 found an 8.3 % infection rate in 60 dogs examined. KHAN 1970 reported that of 442 dogs examined in the Sudan for the presence of Salmonella, 104 (23.5 %) were found to be infected.

The frequency with which Salmonella organisms are isolated in this study from faeces of dogs from three different origins is given in the result. In 59 (25.65 %) from a total of 230 dogs examined for Salmonella infection, 15 different types were isolated. In comparison with the other reports, the percentage of Salmonella infection in dogs in West Berlin is found to be among the highest. The high rate of Salmonella infection in dogs, which was found in this study, supports the work of GOUDSWAARD 1969 who disproved the widely held view based on Swedish, Italian, and English studies thirteen years ago that Salmonella infection in dogs was not very common in Europe.

3. The Epidemiological Significance of Salmonellosis in Dogs

A number of dogs carried Salmonella organisms in their digestive tracts without showing any clinical symptoms. Exposure to the Salmonella organism, of course, does not necessarily lead to clinical disease, but it may remain as latent infection. This latency of infection may be disclosed when the animal is put under stress, for example in dogs with canine distemper or intestinal parasites. In those examined, imported dogs which were kept in pet shops some of the main factors which could possibly provoke symptoms of infection are stress of long road or rail journeys and change in environment and feeding. Infection of young dogs could also derive from contact with latent carriers showing transient excretion of the organism due to the presence of intercurrent disease or some

other activating factors like those mentioned above. In any case, it appears that dogs may serve more frequently as host of *Salmonella* than has been thought in the past.

The types of *Salmonella* isolated here are considered under the aspect of their epidemiological significance. The 15 types of *Salmonella* recognized in this work are serologically similar to those frequently infecting man and other animals. Consequently, an epidemiological relationship may exist between dogs, man, and other animals.

The four most common types of *Salmonella* isolated from human beings in West Berlin are *S. typhimurium*, *S. panama*, *S. brandenburg*, and *S. infantis* (unpublished source from the Public Health Centre Department of Epidemiology in West Berlin). With the exception of *S. infantis* (which was isolated only from two faecal samples of dogs), the same types have been found to be the most common in the dogs which were examined in this study. At the same time, all of the 15 *Salmonella* types isolated from these dogs were also isolated from human beings in different hospitals in West Berlin. This notable similarity in distribution of the *Salmonella* serotypes may serve as evidence that *Salmonella* infections in man and in dogs in the same place (in this case in West Berlin), are either derived from a common source or can spread from these animals to man or possibly vice versa. Not enough evidence was obtained, to conclude that dogs are the common source of human salmonellosis. Their relatively high rate of infection, their close association with man and the fact that some authors have reported dogs to have been responsible for some cases of human infection (especially in children), however, indicate that dogs should also be considered as one of the possible sources of *Salmonella* infection whenever outbreaks in man are being investigated.

VI. SUMMARY

1. For the isolation of *Salmonella* from naturally infected faecal specimens of dogs, the resuscitation method has been found to be superior to direct enrichment of the material in selective broths.
2. Factors and procedures influencing the isolation of *Salmonella*, such as choice of resuscitation medium, selective enrichment broths, incubation temperatures, time of subculture from enrichment broths and choice of selective plating media are discussed.
3. Brilliant-green phenol-red lactose agar was found to be superior to litmus lactose agar for isolating *Salmonella* from naturally infected faecal samples of dogs, enriched in tetrathionate and selenite broths.
4. The method for isolating *Salmonella* from the resuscitated faecal cultures of naturally infected dogs, enriched in potassium tetrathionate-brilliant green-bile enrichment broth incubated at 43° C for 24 hr followed by plating on brilliant-green phenol-red lactose agar, proved to give the best result.
5. Statistical analysis showed that the interactions of enrichment method, enrichment media, temperature of incubation and time of subculture were significant (with significance level of 1 %).
6. Faecal cultures from 59 (25.65 %) of 230 dogs were positive for *Salmonella*. Two types of *Salmonella* were isolated from each of seven dogs.
7. Of the fifteen *Salmonella* types found in dogs, four serotypes represent 74 % of the strains isolated, *S. typhimurium* (51.5 %), *S. panama* (7.5 %), *S. brandenburg* (7.5 %) and *S. enteritidis* (7.5 %). The first three *Salmonella* types are also encountered frequently in man in West Berlin.

Z U S A M M E N F A S S U N G

1. Bei der Isolierung von Salmonellen aus natürlich infizierten Hundekotproben erwies sich die Aktivierungsmethode der direkten Anreicherung des Materials in Selektivnährböden überlegen.
2. Umstände und Verfahren, welche die Isolierung von Salmonellen beeinflussen, wie die Wahl des Aktivierungsmediums, die Selektivanreicherungs-nährböden, die Bebrütungstemperatur, die Bebrütungs-dauer der Anreicherungs-nährböden und die Wahl der selektiven Plattennährböden werden diskutiert.
3. Bei der Isolierung von Salmonellen aus natürlich infizierten Kotproben, die in Tetrathionat- und Selenit-Nährböden angereichert wurden, erwies sich der Brillantgrün-Phenolrot-Laktose-Agar dem Lackmus-Laktose-Agar überlegen.
4. Die Methode der Isolierung von Salmonellen aus aktivierten Kotprobenkulturen von natürlich infizierten Hunden, die in Kalium-Tetrathionat-Brillantgrün-Galle-Anreicherungs-nährboden angereichert, bei 43° C 24 Stunden lang bebrütet und anschließend auf Brillantgrün-Phenolrot-Laktose-Agar verimpft wurden, ergab die besten Resultate.
5. Die statistische Analyse zeigte, daß die Wechselwirkung von Anreicherungs-methode, Anreicherungs-medium, Bebrütungstemperatur und Bebrütungs-dauer der Subkultur signifikant war (signifikant für 1 %).
6. 59 Kulturen von 230 Hundekotproben waren salmonellenpositiv (25,65 %). Aus 7 Hundekotproben wurden jeweils 2 verschiedene Salmonellen-Typen isoliert.
7. Von den 15 Salmonellen-Typen, die bei den Hunden gefunden wurden, repräsentieren 4 Serotypen 74 % aller isolierten Stämme: *S. typhimurium* (51,5 %), *S. panama* (7,5 %), *S. brandenburg* (7,5 %), *S. enteritidis* (7,5 %). Die ersten drei Salmonellen-Typen werden auch häufig bei der Bevölkerung Berlins (West) gefunden.

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Curriculum vitae

was born in Asmara, Eritrea, Ethiopia, on July 16, 1943.

After attending elementary and middle school from 1950 to 1958, I became a student of the Haile-Selassie Secondary School in Asmara where I obtained my school leaving certificate in 1962.

From 1962 to 1963, I was trained to become a middle-school teacher in the Teachers Training College (TTC) in Asmara. After attaining a diploma, I attended the Imperial Ethiopian College of Agriculture in Alemaya. In 1965 while still studying, I was offered a scholarship by the University of Warsaw. In the same year I left Ethiopia for Warsaw, where I attended the School of Veterinary Medicine until 1971. Finishing my final examinations on January 29, 1971, I obtained the diploma of Veterinary Surgeon ("Lekarz Weterynarii").

Since April 1971, I have been working on my doctoral dissertation at the Institute of Veterinary Hygiene, Free University of Berlin.

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